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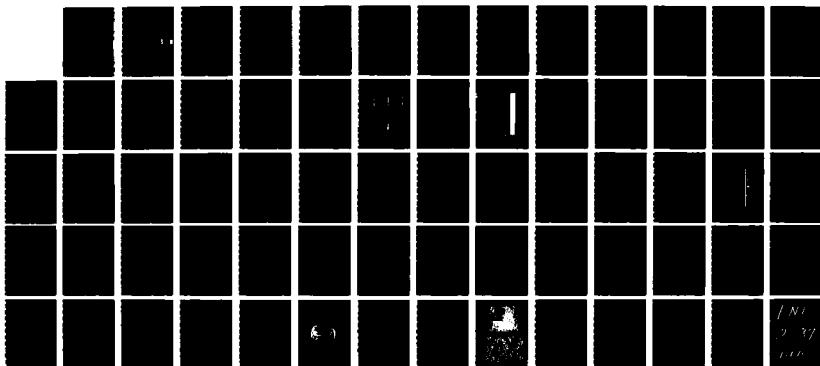
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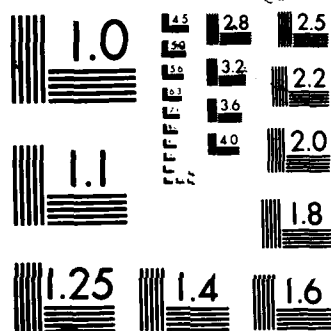
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COMPARISON OF NALOXONE AND  
THYROTROPIN-RELEASING  
HORMONE IN THE TREATMENT  
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ANNUAL REPORT

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COMPARISON OF NALOXONE AND THYROTROPIN-RELEASING HORMONE  
IN THE TREATMENT OF EXPERIMENTAL SPINAL INJURY

Running Title: Endogenous Opioids and Experimental Spinal Injury

ANNUAL REPORT

ALAN I. FADEN

30 SEPTEMBER 1983

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<p>Traumatic injuries to the central nervous system (including spinal cord and brain) cause neurologic impairment not only by directly interrupting neuronal pathways but by initiating a series of pathophysiologic changes which lead to progressive ischemic damage. We have provided evidence that the secondary ischemic changes resulting from experimental spinal trauma are potentially reversible and result, in part, from a reduction of spinal cord blood flow related to the release of endogenous opioids. Previously, we have shown that the opiate receptor antagonist naloxone improves both spinal cord blood flow and neurological outcome following experimental traumatic spinal cord injury in the cat. Subsequently, we found that thyrotropin-releasing hormone (TRH), which acts in part as a physiologic antagonist of endogenous opioid systems, also significantly improves blood flow and neurological recovery after experimental spinal injury. During the first year of the contract we have evaluated the effects of TRH and naloxone against corticosteroids and saline-treated controls. Both naloxone and TRH proved significantly superior to either</p>					
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Because of the increased expenses for cats, particularly the pathogen-free variety, we have developed an alternative traumatic injury model in the rat. This model utilizes the Allen method in which a fixed weight is dropped a fixed distance onto the spinal cord. However, in the rat, as compared to the cat, the injury site is in the lower thoracic region. We have now established dose-response studies for injury with relatively complete, long-term paraplegia produced by injuries caused by 75-100 g-cm impact energy.

Finally, in order to determine which endogenous opioid system and which receptor population must be responsible for the pathophysiological effects following spinal cord injury, we compared the effects of a variety of endogenous opioids and synthetic endorphins following intrathecal administration in the rat. Only the dynorphin family of opioids, which are active at the  $\kappa$ -opiate receptor, produced paralysis in this model. In contrast, synthetic enkephalins, which act at either the  $\mu$  or  $\delta$  receptor, and  $\beta$ -endorphin, which acts at the  $\epsilon$  receptor, failed to produce such changes.

The present findings suggest that the dynorphin family of endogenous opioids may be involved in the pathophysiology of spinal cord injury: these effects may result from actions at the  $\kappa$ -opiate receptor, and they may involve changes in local microcirculation. TRH, whose mechanism of action is different from that of naloxone, has proved therapeutically superior in traumatic injury models. The beneficial effects of TRH are found even after relatively low doses and even relatively late (24 h) after injury.

## SUMMARY

Traumatic injuries to the central nervous system (including spinal cord and brain) cause neurologic impairment not only by directly interrupting neuronal pathways but by initiating a series of pathophysiologic changes which lead to progressive ischemic damage. We have provided evidence that the secondary ischemic changes resulting from experimental spinal trauma are potentially reversible and result, in part, from a reduction of spinal cord blood flow related to the release of endogenous opioids. Previously, we have shown that the opiate receptor antagonist naloxone improves both spinal cord blood flow and neurological outcome following experimental traumatic spinal cord injury in the cat. Subsequently, we found that thyrotropin-releasing hormone (TRH), which acts in part as a physiologic antagonist of endogenous opioid systems, also significantly improves blood flow and neurological recovery after experimental spinal injury. During the first year of the contract we have compared the effects of TRH and naloxone against corticosteroids and saline-treated controls. Both naloxone and TRH proved significantly superior to either saline or high-dose corticosteroids in improving long-term, functional neurological recovery in the cat. Moreover, TRH proved significantly better than naloxone in this regard. In separate studies we found that corticosteroids (including either dexamethasone or methylprednisolone), even in the megadose range, failed to improve neurological recovery in this traumatic cat model. Subsequently, we completed independent studies showing that the therapeutic effects of TRH were clearly dose-related, with beneficial actions observed at doses as low as 0.02 mg/kg. Of particular importance, TRH treatment significantly improved neurological recovery even when the drug was not administered until fully 24 h after traumatic injury.

Because of the increased expenses for cats, particularly the pathogen-free variety, we have developed an alternative traumatic injury model in the rat. This model utilizes the Allen method in which a fixed weight is dropped a fixed distance onto the spinal cord. However, in the rat, as compared to the cat, the injury site is in the lower thoracic region. We have now established dose-response studies for injury with relatively complete, long-term paraplegia produced by injuries caused by 75 - 100 g-cm impact energy.

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# FOREWORD

Citations of trade names in this report do not constitute an official Department of the Army endorsement or approval of the use of such items.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council (DHEW Pub. No. [NIH] 78-23, Revised 1978).

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This is the first annual report submitted under contract #USAMRDC 01120-82 and covers the period 1 August 1982 - 31 July 1983. The studies reported include: (1) comparison of the beneficial effects of TRH, naloxone and corticosteroids following experimental spinal cord injury in the cat; (2) effect of megadose corticosteroids (both dexamethasone and methylprednisolone) in this same injury model; (3) independent studies examining the dose-response effects of TRH on spinal cord injury in the cat, as well as the effects of late (24 h post-injury) treatment; (4) preliminary studies establishing a spinal trauma model in the rat; and (5) intrathecal infusions of selective endogenous opioids and opiate agonists to assess the potential role of specific opioids and/or opiate receptors in the paralysis which follows traumatic injury.

Problem. It is clear that much of the neurological deficit which follows traumatic injury to the central nervous system results not from the immediate and direct effects of the trauma in severing neuronal connections, but rather from secondary responses initiated by the injury which result, in part, from reductions of blood flow due to release of endogenous factors. It has previously been established that endogenous opioid systems contribute to the secondary pathophysiological events by contributing to the reduction in spinal cord blood flow. Both the opiate antagonist naloxone and the physiologic opiate antagonist TRH have been shown to be effective in a model of traumatic spinal cord injury in the cat. The purpose of the present studies was to compare naloxone and TRH against one another and to other experimental therapies; to establish the dose-response characteristics and the effects of time of treatment of TRH in experimental spinal injury; to determine which endogenous opioids and which opiate receptor systems might play a role in the

pathophysiologic response in spinal cord injury; and finally to establish a less costly, predictive, traumatic, spinal injury model in the rat.

#### BACKGROUND

Traumatic injuries to the spinal cord may cause neurologic impairment in two ways - by directly interrupting neuronal pathways and/or by initiating a series of pathophysiologic changes which lead to progressive ischemic damage to the spinal cord (1,2). There is experimental evidence that these ischemic changes are potentially reversible and result, at least in part, from a reduction of spinal cord blood flow (SCBF) (3-6). We have postulated that spinal trauma activates endorphin systems which contribute to post-traumatic reduction of spinal blood flow and that treatment with opiate antagonists, by reversing the effects of endorphins, improves SCBF and neurological outcome. We have tested this hypothesis, using a cat spinal injury model (7,8). Experimental spinal cord injury did cause an elevation of plasma  $\beta$ -endorphin immunoreactivity associated with a reduction in SCBF; naloxone treatment significantly improved SCBF as compared with saline controls (8). More critically, naloxone-treated animals showed significantly better neurological recovery over a three-week follow-up period: the average naloxone-treated animals walked well with minimal spasticity, whereas the average saline control animals was unable to walk without support. In addition, there was a lower mortality rate in naloxone-treated animals than in saline controls. We later replicated and extended these findings in a more severe spinal injury model; again naloxone-treated animals showed significantly greater functional neurological recovery than saline controls as late as six weeks following the spinal injury (9).

In the latter study naloxone administration was shown to improve neurological recovery even if treatment was not instituted until four hours following spinal injury.

Subsequently, we demonstrated that thyrotropin-releasing hormone (TRH), which appears to act in vivo as a partial "physiological opiate antagonist," also markedly improves functional neurological recovery after spinal injury (10). Moreover, we have shown that TRH is superior to naloxone in improving SCBF after traumatic injury in the rat (11). A potential advantage of TRH over naloxone is that TRH does not affect pain sensitivity, whereas naloxone, by blocking the analgesic effect of endogenous opioids, could potentially exacerbate post-traumatic pain states.

The purpose of the present studies, therefore, was: (1) to directly compare naloxone and TRH against one another and against treatment with high-dose corticosteroids, which is considered the treatment of choice for human spinal cord injury in many spinal centers; (2) to determine the effects of treatment dose and time of treatment on the therapeutic actions of TRH; (3) to determine whether megadose corticosteroids, currently proposed by investigators as superior to even high-dose corticosteroid treatment, compares to treatment with either naloxone or TRH. In addition, because of the very high cost of the cat trauma model, which relies on pathogen-free animals, we have attempted to establish a similar traumatic spinal cord model in the rat.

Finally, the aims of the present studies were to further address the issue of which endogenous opioids and which opiate receptor systems might play a role in the pathophysiological process which follows traumatic injury.

## MATERIALS AND METHODS

Details regarding the materials and methods utilized in the studies during the first contract year are provided in a number of accompanying manuscripts, either published, in press or in review, which are appended. Briefly, three series of studies have been performed. The cat trauma model utilizes pathogen-free cats which are anesthetized with pentobarbital and paralyzed. A 20 gm weight is dropped a distance of 30 cm through a guide tube onto the exposed spinal cord. At a fixed time following injury (from 1 - 24 h), treatment is begun as a bolus, i.v. injection followed by i.v. infusion over 4 h. Following removal of catheters, animals are returned to home cages where they are evaluated in blinded fashion by two investigators; neurological scores are rated on a 10-point ordinal scale, as previously described, based on motor function. Subsequently, animals are killed and the spinal cords processed and examined for histopathological changes. The rat trauma studies are very similar, except that animals are anesthetized with a combination of pentobarbital and ketamine, and the site of injury is at T-10 as compared with C-7 in the cat. Injury parameters in the rat vary from 25 g-cm to 100 g-cm, the latter producing complete paraplegia in animals as long as one month following injury. Intrathecal infusion studies in the rat utilized a slight variation of the method by Yaksh and Rudy (12). This method is detailed separately (13) and permits intrathecal infusions through PE 10 tubing in the awake, freely moving rat. Equal volume infusions are administered utilizing highly selective opioids or opiate agonists.

Pharmacological studies in the cat utilize naloxone at a dose of 2 mg/kg bolus, followed by 2 mg/kg/h. TRH doses range from 0.02 mg/kg to 2 mg/kg, each

given as i.v. bolus, followed by 4 h i.v. infusion. Dexamethasone is given in a similar manner at doses from 0.5 mg/kg up to 14 mg/kg. In contrast and in keeping with other studies, methylprednisolone is given intravenously (first dose), followed by t.i.d. declining doses over a 10 day post-operative period.

## RESULTS

In the cat spinal injury model, both naloxone and TRH therapies proved significantly superior to either high-dose corticosteroids or to saline controls (Fig. 1). At six weeks post-injury the median naloxone animal showed normal function in forelimbs and only mild spasticity in hindlimbs; the median TRH animal appeared to be entirely normal, whereas animals treated with corticosteroids or saline were indistinguishable from one another and demonstrated severe spastic quadriparesis. In this model, megadose corticosteroids utilizing up to 30 mg/kg of methylprednisolone, and up to 14 mg/kg of dexamethasone, proved ineffective and, indeed, somewhat increased mortality rates (Fig. 2). In independent studies, TRH proved statistically superior to saline controls in a dose-related manner (Fig. 3). Although optimal effects were observed with the highest dose utilized (2 mg/kg), significant beneficial effects on motor recovery were observed with even relatively low doses (0.02 mg/kg). Remarkably, TRH, at high doses, proved effective even when administered as late as 24 h after injury (Fig. 3). Combination treatment with naloxone and TRH did not prove to be superior to treatment with TRH alone.

During the contract year we have successfully established a traumatic thoracic spinal cord injury in the rat, based on the same Allen method technology

as used in the cat. Injury parameters of 25 g-cm, 50 g-cm, 75 g-cm and 100 g-cm applied to the T-10 region in laminectomized rats produced dose-related paraparesis at four weeks post-injury (see Table 1).

Finally, intrathecal infusion studies demonstrated that the dynorphin family of endogenous opioids, unique amongst opioids, produced dose-related and partially reversible hindlimb in the rat (Fig. 4). In contrast, a variety of synthetic enkephalins, active at the  $\mu$ - and  $\delta$ -opiate receptors, as well as  $\beta$ -endorphin, which is active at the  $\epsilon$  receptor, failed to produce any change in motor function, even at doses significantly higher than those used for dynorphin. The most potent effects in the dynorphin family were observed with the native ligand (dynorphin 1-17).

#### CONCLUSIONS

The present studies demonstrated that both TRH and naloxone significantly improve long-term motor recovery following traumatic spinal cord injury in the cat. Both therapies are superior to either high-dose or megadose corticosteroids, which have long been considered the treatment of choice in human spinal cord injury. In studies directly comparing the effects of naloxone and TRH, TRH proved significantly better than naloxone, with the average animal showing essentially complete recovery at six weeks following traumatic injury. The beneficial effects of TRH on motor recovery were clearly dose-related, with beneficial effects observed with doses as low as 0.02 mg/kg. Of great importance was the somewhat unexpected observation that TRH treatment, at high doses, was effective in promoting neurological recovery, even



when treatment was delayed as long as 24 h following injury. Taken together, the present animal studies provide the basis for a therapeutic trial for TRH in human spinal cord injury.

In addition, experimental studies in the rat provided evidence to suggest that the endogenous opioid dynorphin, which may be the endogenous ligand for the  $\kappa$ -opiate receptor, may be the pathophysiological endorphin in spinal cord injury. Dynorphin-related peptides, unique amongst opioid peptides or synthetic enkephalins, caused dose-related hindlimb paralysis in the rat. This finding suggests that therapies which are directed at dynorphin more specifically, or at the  $\kappa$ -opiate receptor, may have even a higher degree of selectivity as therapeutic agents in traumatic spinal cord injury.

#### RECOMMENDATIONS

It is our intention to continue and extend the present findings by evaluating the beneficial effects of highly selective opiate receptor antagonists in the cat and rat models of traumatic spinal cord injury. In addition, we will begin pilot studies examining the beneficial effects of long-acting and highly potent TRH-analogs from Europe.

The present studies, combined with previous studies from our laboratory, provide the experimental basis for clinical trials of TRH in human spinal cord injury. Phase I studies have already begun at the University of California, San Diego. If TRH proves safe at doses which are considered necessary in the treatment of spinal cord injury, multi-institutional Phase II and Phase III studies will be initiated.

TABLE 1

RAT MOTOR FUNCTION AT 1 WEEK FOLLOWING TRAUMATIC INJURY

Neurological Score	Impact Energy (g-cm)				
	10	25	50	75	100
0	---	---	---	xxx	xx
1	xxx	xx	xxxxxxxx	xxxxxxxxxx	xx
2	xx	x	x	---	---
3	xxxx	---	x	---	---

Neurological scores, as follows: 0 = no movement; 1 = spontaneous movement, unable to walk; 2 = able to walk with marked spasticity or ataxia; and 3 = normal walking. x = individual animal scores.

## FIGURES

Figure 1. Effect of TRH (T), naloxone (N), dexamethasone (D) and saline (S) treatments on neurologic recovery after traumatic cervical spinal cord injury in cats. Both TRH- and naloxone-treated animals showed significantly higher neurologic scores than saline controls over the six-week follow-up period. Moreover, scores in TRH animals were significantly higher than either naloxone or dexamethasone animals at six weeks post-injury. Points represent the sum of forelimb and hindlimb neurologic scores for individual animals; histograms represent median scores.

Figure 2. Effects of methylprednisolone (n=6) or saline (n=8) treatment on neurologic recovery six weeks after cervical spinal cord injury. Points represent the sum of forelimb and hindlimb neurologic scores for individual animals; histograms represent median scores. No significant differences were observed between the groups.

Figure 3. Effects of thyrotropin-releasing hormone or saline treatment on neurologic recovery six weeks after cervical spinal cord injury in cats. TRH treatment significantly improved motor recovery in a dose-related manner. TRH also significantly reduced late paralysis, even when treatment was not administered until 24 h after injury. Points represent the sum of forelimb and hindlimb neurologic scores for individual animals; histograms represent median scores.

Figure 4: A. Dose-response effects of DYN 1-13 on motor function in the rat following intrathecal administration. DYN 1-13 produces dose-related, partially reversible paralysis of hindlimb function, with peak effects at approximately 50 nmol.

B. Comparison of effects of DYN 1-17, DYN 1-13, DYN 1-8 and  $\alpha$ NE motor function in the rat. Each peptide produces hindlimb dysfunction, with the order of potency being DYN 1-17 > DYN 1-13 >>  $\alpha$ NE  $\approx$  DYN 1-8.

# NEUROLOGICAL RECOVERY

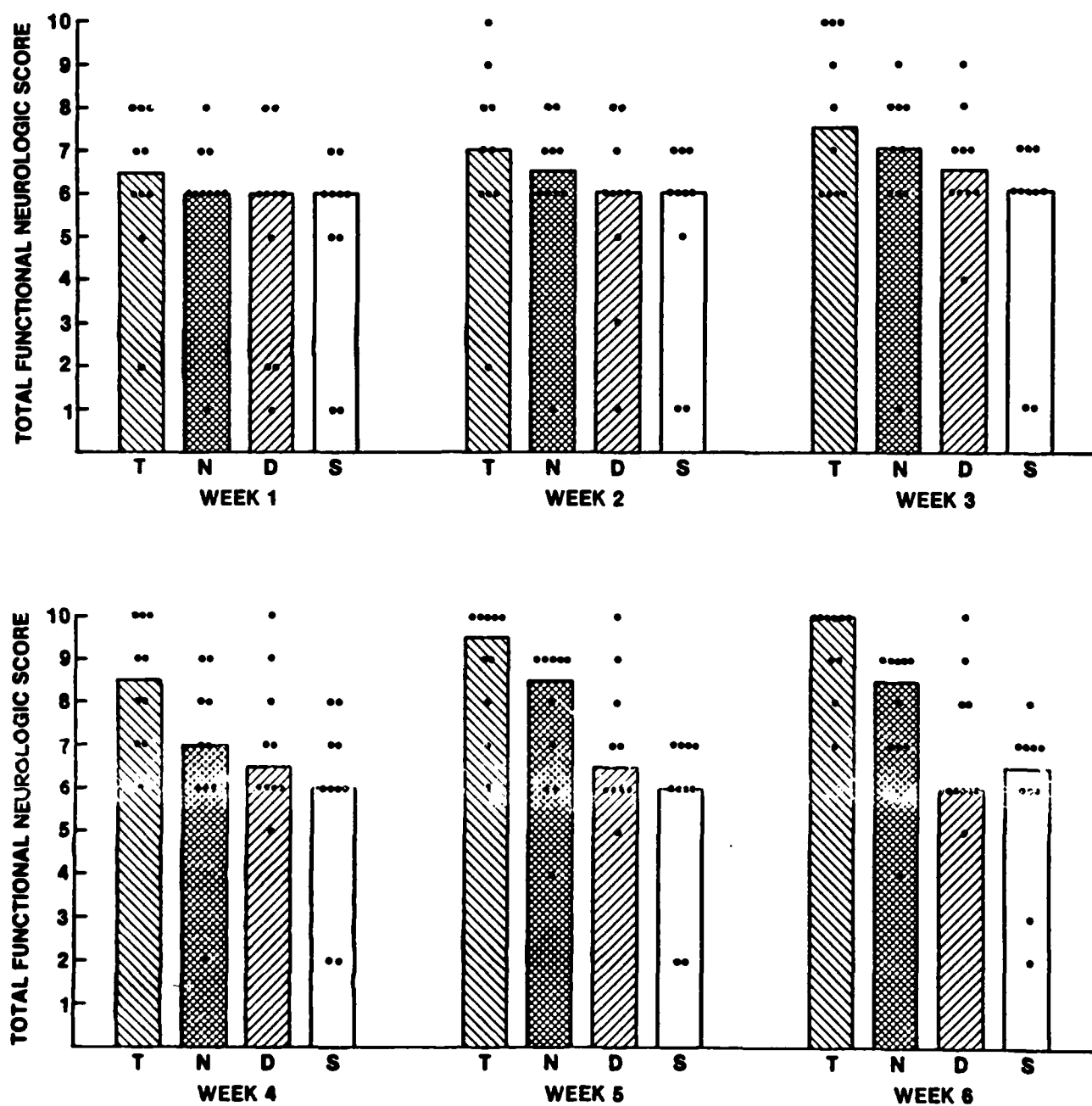


FIGURE 1

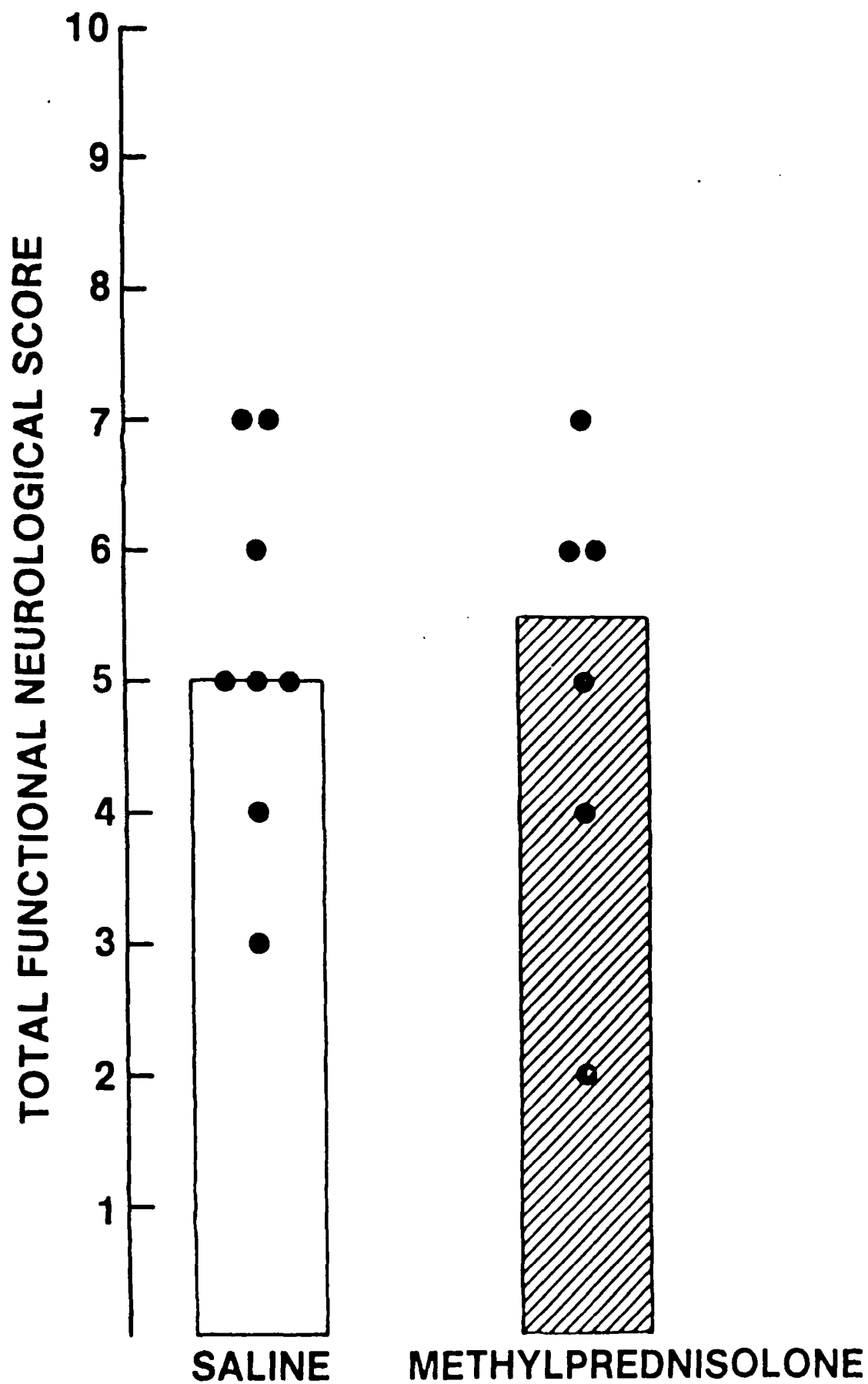


FIGURE 2

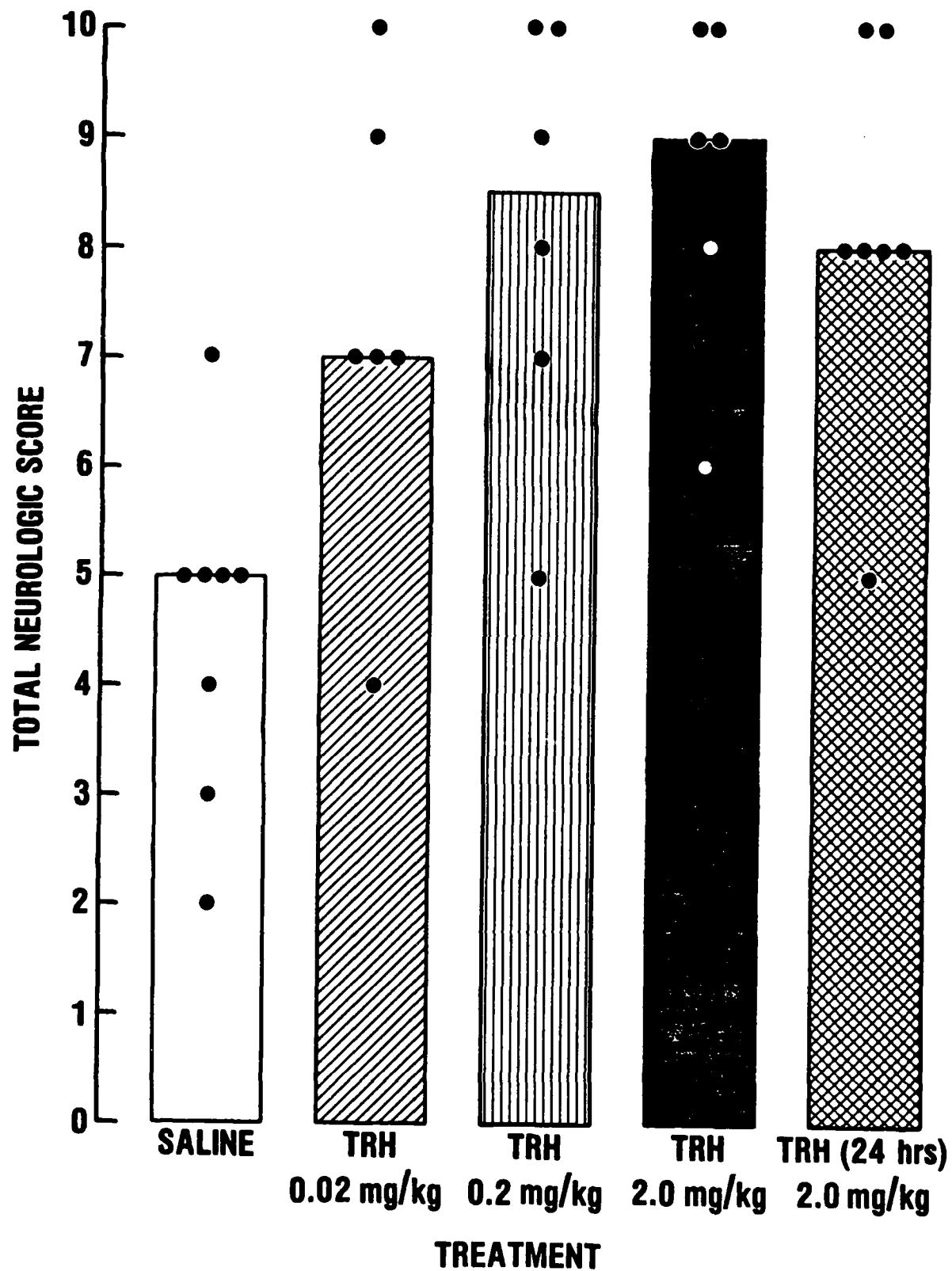
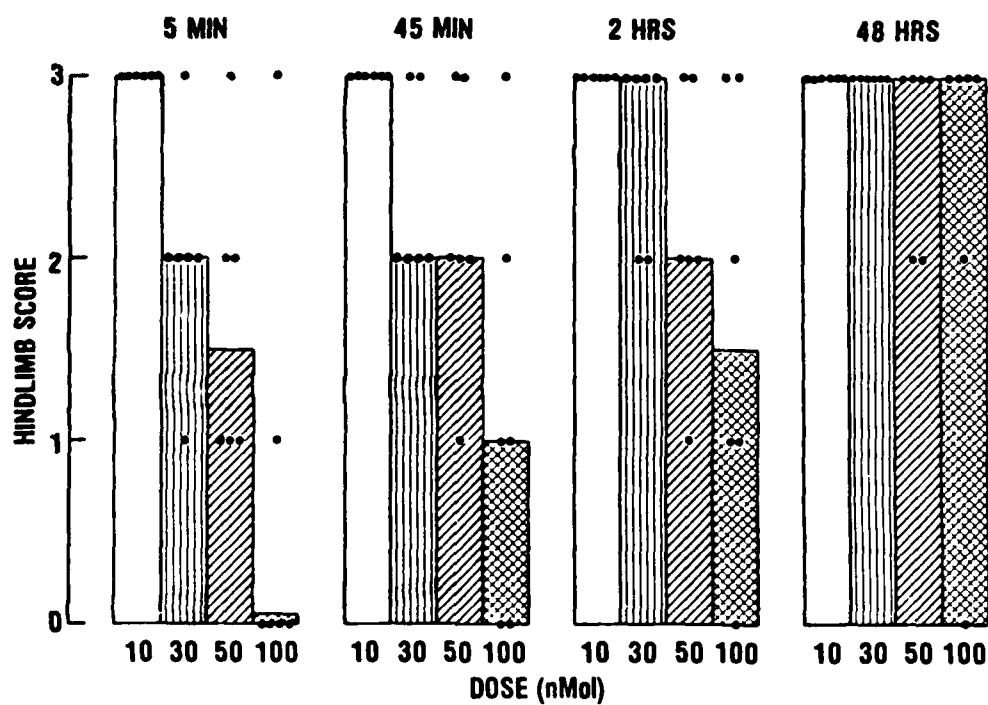


FIGURE 3

# A. TIME AFTER INJECTION



# B. 15 MINUTES AFTER INJECTION

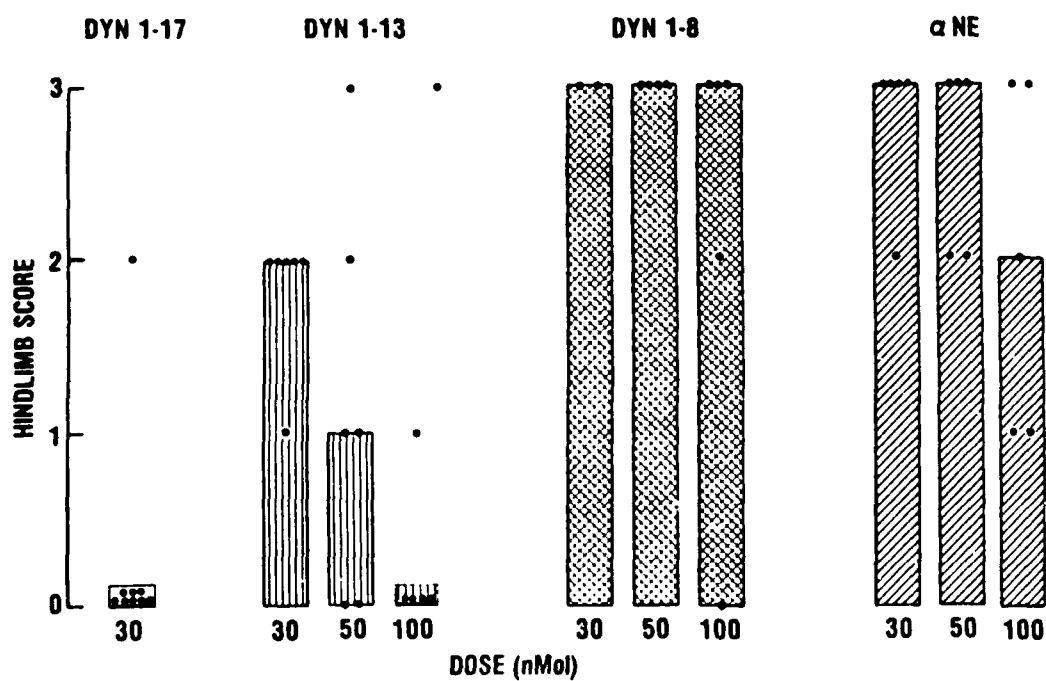


FIGURE 4



#### LITERATURE CITED

1. Ducker TB, Kindt GW, Kempe LG: Pathological findings in acute experimental spinal cord trauma. J Neurosurg 35:700-708, 1971.
2. Osterholm JL: The pathophysiological response to spinal cord injury. J Neurosurg 40:5-33, 1974.
3. Sandler AN, Tator CH: Review of the effect of spinal cord trauma on the vessels and blood flow in the spinal cord. J Neurosurg 45:638-646, 1976.
4. Dohrmann GJ, Wick KM, Bucy PC: Spinal cord blood flow patterns in experimental traumatic paraplegia. J Neurosurg 38:52-58, 1973.
5. Ducker TB, Perot PL: Spinal cord oxygen and blood flow in trauma. Surg Forum 22:413, 1971.
6. Ducker TB, Salzman M, Lucas JT, Garrison WB, Perot PL: Experimental spinal cord trauma. II: Blood flow, tissue oxygen, evoked potentials in both paretic and plegic monkeys. Surg Neurol 10:64-70, 1978.
7. Faden AI, Jacobs TP, Holaday JW: Opiate antagonist improves neurologic recovery after spinal injury. Science 211:493-494, 1981.
8. Faden AI, Jacobs TP, Mougey E, Holaday JW: Endorphins in experimental spinal injury: Therapeutic effect of naloxone. Ann Neurol 10:326-332, 1981.
9. Faden AI, Jacobs TP, Holaday JW: Comparison of early and late naloxone treatment in experimental spinal injury. Neurology 32:677-681, 1982.
10. Faden AI, Jacobs TP, Holaday JW: Thyrotropin-releasing hormone improves neurologic recovery after spinal trauma in cats. N Engl J Med 305:1063-1067, 1981.

11. Faden AI, Jacobs TP, Smith GP, Green B, Zivin J: Neuropeptides in spinal cord injury: Comparative experimental models. Winter Neuropeptide Conference, Peptides (In Press), 1983.
12. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. Physiol Behav 17:1031-1036, 1976.
13. Faden AI, Jacobs TP: Dynorphin induces partially reversible paraplegia in the rat. Eur J Pharmacol 91:321-324, 1983.

APPENDIX A

THYROTROPIN-RELEASING HORMONE IN  
EXPERIMENTAL SPINAL INJURY: BENEFICIAL EFFECTS  
OF LOWER DOSE TREATMENT AND LATE TREATMENT

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## ABSTRACT

Early treatment with thyrotropin-releasing hormone (TRH) at high doses improves neurological recovery following experimentally-induced spinal cord injury, and is significantly better than treatment with naloxone or high dose corticosteroids, two therapies currently proposed for human spinal injury. In the present studies we compared treatment with high dose, intravenous TRH (2 mg/kg bolus followed by 2 mg/kg/hr over four hrs) against two lower doses (0.2 mg/kg and 0.02 mg/kg, each bolus dose plus four hr infusion), in order to establish the optimal treatment dose for spinal cord injury. Six animals each received TRH at one of the three doses; eight cats received equal volume infusions of physiological saline. Treatment was begun one hr post-trauma, with cervical spinal injury produced by the Allen (weight-drop) method in anesthetized animals. An additional seven cats received high dose TRH treatment beginning 24 hrs post-trauma. Cats treated with TRH showed significantly enhanced motor recovery as compared with saline controls; effects were dose related, with optimal effects observed at the higher doses. Cats treated at 24 hrs post-injury also showed significantly improved functional recovery. These findings provide further experimental support for the institution of TRH trials in human spinal injury and indicate that even relatively late treatment may be effective. Moreover, the effectiveness of TRH at 24 hrs post-injury underscores the plasticity of mammalian central nervous system and indicated that classical concepts regarding the pathophysiological basis of neurologic dysfunction after spinal injury may need to be modified.

## INTRODUCTION

The neurological dysfunction caused by traumatic spinal injury results only in part from direct disruption of spinal nerve cells or fibers. Rather, the loss of function appears to result from delayed, secondary pathophysiological mechanisms initiated by the trauma which develop following injury (1). This concept of indirect, delayed damage has provided the rationale for physical (e.g., hypothermia) or pharmacological interventions, whose purpose is to reverse or modify such secondary injury.

Several years ago we demonstrated that endogenous opioids were released following experimental spinal injury (2) and suggested that they served as pathophysiological factors, contributing to the secondary injury process by reducing spinal cord blood flow (SCBF) (2,3). Treatment with the opiate receptor antagonist naloxone improved SCBF (2) and enhanced functional neurological recovery (2-4). However, non-selective opiate antagonists like naloxone may have the adverse effect of exacerbating post-traumatic pain, since they block the analgesic effect of opioids.

Thyrotropin-releasing hormone (TRH) is a tripeptide which is widely distributed in the central nervous system and which possesses a wide variety of physiological actions (5). Among these is its ability to act in vivo as a partial physiological opiate antagonist, but relatively unique in that it reverses the autonomic effects of opioids without altering their analgesic actions (6,7). For these reasons we evaluated TRH treatment in a feline model of cervical spinal cord injury (8). Animals treated with

TRH at one hr post-trauma showed a remarkable degree of functional recovery, with the average cat appearing entirely normal at six weeks post-injury; in contrast, animals treated with either physiological saline or dexamethasone had severe spastic quadriparesis (8). In this cervical injury model, TRH treatment has proved significantly better than treatment with naloxone or higher dose corticosteroids (9), two therapies currently proposed for treatment of human spinal injury.

The dose of TRH used in previous spinal injury studies (2 mg/kg bolus, followed by 2 mg/kg/hr over four hrs) is orders of magnitude higher than doses of the peptide used in humans for endocrine evaluation (10); such doses were based on earlier work from our laboratory in the area of experimental shock (7). Moreover, in earlier studies TRH infusions were begun shortly after trauma (one hr), whereas patients rarely reach treatment facilities within the first four hrs following injury. The purposes of the present study, therefore, were to establish the optimal treatment dose of TRH in spinal cord injury, as well as to determine whether relatively late treatment might be therapeutically effective.

#### METHODS

Cats (specific pathogen-free, Liberty Labs, 2.5 - 3.5 kg) were anesthetized with pentobarbital (30 mg/kg of body weight). After an endotracheal tube was placed, animals were paralyzed with gallamine triethiodide and ventilated using a Harvard respirator.

Arterial blood gas values were kept in the normal range by adjusting the respirator and through the use of intravenous sodium bicarbonate. A feedback thermoregulatory unit was utilized to maintain temperature at 38°C. Blood pressure was continuously recorded from a femoral-artery catheter utilizing a Narco pressure transducer (RP15001) attached to a Narcotrace physiograph (NT-80). A catheter in the femoral vein permitted drug administration. After the animals were fixed in a stereotaxic spinal unit (Kopf), the C7 spinal segment was exposed under aseptic conditions through a laminectomy. The C7 spinal segment was injured utilizing a modification of the Allen method in which a 20 g weight was dropped 30 cm through a guide tube onto a 10-mm<sup>2</sup> impact plate, resting on the exposed, intact dura mater (600 g-cm impact energy) (8). These injury variables have been utilized previously in our laboratories since they produce moderately severe but incomplete spinal cord injury, in which control animals show severe spastic quadriparesis at six weeks post-injury (8). In 26 animals, treatment was begun one hr post-trauma, drugs being given intravenously as bolus injection (0.5 ml), followed by continuous infusion at 0.5 ml/hr over four hrs utilizing a Harvard infusion pump. These early treatment animals were assigned to one of four treatment regimens: (1) TRH (2 mg/kg as a bolus, and 2 mg/kg/hr, n=6); (2) TRH (0.2 mg/kg as a bolus, and 0.2 mg/kg/hr, n=6); (3) TRH (0.02 mg/kg as a bolus, and 0.02 mg/kg/hr, n=6); or (4) physiologic saline (n=8). After treatment, the laminectomy site was closed, the catheters were removed and the animals were moved to an intensive

care unit where humidity and ambient temperature could be controlled. An additional seven animals were subjected to the same experimental protocol and injury variables, but were administered TRH (2 mg/kg as a bolus, and 2 mg/kg/hr, n=7) through a superficial limb vein at 24 hrs post-injury. A neurologist, unaware of treatment group, graded neurological function weekly using a five-point ordinal scale. Motor function was rated in forelimbs and hindlimbs separately as follows: 0, absence of voluntary movement; 1, spontaneous movement but inability to support weight; 2, ability to support weight but not to walk; 3, ability to walk but with marked spasticity or ataxia or both; 4, ability to run but with mild spasticity or ataxia; and 5, normal motor function. Addition of the forelimb and hindlimb scores yielded a total functional neurological score. At six weeks post-injury, animals were killed with intravenous pentobarbital for histopathological examination.

Spinal cords were removed and fixed in refrigerated 2% glutaraldehyde for seven days. Spinal cord segments C5-T2 were serially sliced in cross-section at 2 mm intervals allowing careful measurement of the craniocaudal extent of lesions present. The cross-sectional slices were then dehydrated in graded alcohols, imbedded in paraffin and cut in 5 $\mu$  sections with a rotary microtome. Sections were stained with hematoxylin, eosin and luxol-fast blue and studied with a light microscope. Spinal cord sections were evaluated in blinded fashion. The total area of demyelination at the injury site was measured using an ocular micrometer. Total volume of injury was calculated using the



formula  $V = L \times X \times Y$ ; where V is volume; L is craniocaudal extent of injury; X and Y are the sides of the rectangle approximated by the cross-sectional area of injury. The volume of injury was scored according to the following criteria:

<u>Score</u>	<u>Injury Volume (mm<sup>3</sup>)</u>
1	81 - 100
2	61 - 80
3	41 - 60
4	21 - 40
5	0 - 20

Differences in neurological scores and histopathological scores among the groups were compared utilizing Kruskal-Wallis analysis of variance followed by individual Mann-Whitney Rank-Sum Tests. Neurological and histopathological scores were compared using the Spearman Rank Correlation Test. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

### Neurological Function

At six weeks post-trauma, total functional neurological scores were significantly higher in TRH-treated animals (Figure 1) than in saline controls (Kruskal-Wallis Stat = 14.73,  $p = 0.005$ ). Moreover, significantly enhanced motor recovery was found in each TRH treatment group as compared with controls: TRH (2.0 mg/kg)  $p < 0.001$ ; TRH (0.2 mg/kg)  $p < 0.005$ ; TRH (0.02 mg/kg)  $p < 0.025$ ; TRH (2.0 mg/kg, 24 hr)  $p < 0.005$ . TRH effects on motor recovery were dose-related, although only modest differences were observed

between the high dose (2 mg/kg) and moderate dose (0.2 mg/kg) treated animals. Among animals treated with the high TRH dose, only minimal differences were noted between animals treated one hr post-injury and those whose treatment was not begun until 24 hrs post-injury. Neurological scores in TRH-treated animals exceeded those treated with saline as early as one week post-trauma; these differences increased during the remaining weeks of observation (Table 1).

#### Histopathology

The spinal cord sections displayed typical traumatic lesions after the standardized trauma: many were cavitated; all showed degrees of gliosis, demyelination and axonal reaction. Remaining myelin was concentrated subpially at the periphery of the cord sections. Minimal mononuclear cellular reaction was present. Mean cross-sectional area of lesions and mean length of lesions for grades 1 - 5, respectively, were: 23.78 mm<sup>2</sup>, 4.35 mm; 21.18 mm<sup>2</sup>, 3.37 mm; 20.10 mm<sup>2</sup>, 2.45 mm; 19.22 mm<sup>2</sup>, 1.89 mm; 13.30 mm<sup>2</sup>, 1.0 mm. However, histopathological scores between TRH- and saline-treated animals did not differ significantly (Kruskal-Wallis Stat = 8.10, p = 0.88), although scores for early, high-dose TRH animals (Table 2) were higher than those in the other TRH or control groups. Clinical pathological correlations, moreover, were not significant for any treatment group, even when all treatment groups were combined (Spearman Rank Correlation Test, p > 0.05).

## DISCUSSION

The findings in this study show that thyrotropin-releasing hormone can improve functional neurological recovery following experimental spinal injury at doses as low as 0.02 mg/kg (bolus plus infusion; total dose 0.1 mg/kg). This dose is one one-hundredth of that shown to be effective in our previous studies and approaches the range of doses of thyrotropin-releasing hormone routinely utilized in humans (10). Optimal effects were noted at higher doses with little apparent difference between animals treated with 2.0 mg/kg or 0.2 mg/kg. Although the latter TRH dose is high when compared with that utilized in most endocrinological studies, it is lower than the intravenous doses used in other experimental clinical studies (11,12). Recently, Engel and colleagues have shown that intravenous infusions of thyrotropin-releasing hormone are well tolerated by patients with severe motor dysfunction in doses as high as 19 mg/min or 500 mg/day (12). These authors observed that TRH treatment at very high doses (> 10 mg/min) resulted in substantial improvement in muscle strength and reduction of spasticity in patients with long-standing, severe amyotrophic lateral sclerosis (12). Although use of high TRH doses in this clinical study was based on our experimental spinal studies, the results differed in one critical regard: namely, the beneficial effects in patients with amyotrophic lateral sclerosis were transitory and contingent upon the maintenance of high TRH levels, whereas in the cat trauma studies TRH infusion resulted in functional improvement which was sustained.

The finding that TRH treatment improves functional recovery, even when such treatment is administered fully 24 hrs post-trauma, is of substantial theoretical and therapeutic importance. Spinal-injured patients rarely reach medical treatment facilities less than four hrs following trauma. In contrast to virtually all other experimental, pharmacological therapies, whose effectiveness have been demonstrated only when administered in the first hours post-injury, the beneficial effects of late TRH treatment indicate that it might have therapeutic application to virtually all patients with acute spinal injury.

The mechanism by which TRH exerts its therapeutic effects in experimental spinal injury remains speculative. Although we originally proposed treatment with TRH because it appeared to be a physiologic antagonist of endogenous opioid systems (8), this explanation appears unlikely to account for all of its therapeutic action, since we have shown that TRH treatment is statistically superior to that of naloxone (9). TRH, like naloxone, appears to have beneficial effects on spinal cord blood flow following traumatic injury and may be superior to naloxone in this regard (13). Moreover, TRH but not naloxone significantly reverses some of the pathophysiological effects of certain recently described vasoactive lipids (such as leukotrienes and platelet-activating factor) (14-16), substances which may play a pathophysiological role in traumatic central nervous system injury.

Immunoreactive TRH is found in the spinal cord, particularly within the ventral horn (17,18), as are TRH receptors (19). Most

of this TRH appears to be derived from supraspinal sources (possibly in the raphe area); and may be colocalized with substance P and 5-hydroxytryptamine (20). Levels of immunoreactive TRH are markedly reduced following spinal cord transection (18) and may be reduced substantially following spinal cord injury (unpublished observation). Neurophysiological studies have shown that TRH has potent depolarizing actions on motoneurons and increase the excitability of motoneurons (21,22). Moreover, TRH increases the amplitudes of monosynaptic and polysynaptic-evoked potentials within the spinal cord (23). Taken together, these findings suggest that alterations in TRH following spinal cord injury could contribute to functional changes observed after trauma. However, these findings do not appear to provide adequate explanation for the observation that functional changes are permanently altered by short-term intravenous infusions of TRH. A more persuasive explanation might be that acute spinal cord injury alters the critical balance between neurotransmitters, neuromodulators and certain classes of receptors; this balance may be particularly sensitive to pharmacological manipulation, partly due to the loss of functional reserve resulting from loss of neural input (e.g. of supraspinal origin). However, independent of this hypothesis, the present findings clearly underscore the fact that despite the severe functional deficit following spinal cord injury, certain descending pathways remain physically intact and potentially capable of resuming normal function. TRH is clearly able to facilitate this potential plasticity.

The failure to observe significant differences in histopathological scores between TRH-treated and saline control animals, despite the substantial functional differences confirms earlier work by us (9), as well as by others (22). Such a finding suggests either that light microscopic evaluation is insufficiently sensitive to detect existing pathological differences, or that the neurological deficit in control animals results in part from more functional alterations (e.g., in modulators or receptors), as proposed above.

A large variety of pathophysiological factors have been implicated in spinal cord injury including free radicals (25), monoamines (26), endogenous opioids (27), changes in calcium flux (28), changes in blood flow (29), changes in lipid-dependent enzymes ( $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ ) (30), etc. However, in virtually all hypotheses regarding the pathophysiology of spinal cord injury, it has been assumed that irreversible effects occur by four to eight hrs post-injury (31). Similar conceptualizations underscore current theories regarding the pathophysiology of head injury and the pathophysiology of cerebral ischemia (32). In contrast, the present finding that pharmacological treatment 24 hrs post-injury can reverse the functional deficit, even to the point of normal return of function, must cause a reconsideration of such classical concepts. It appears that substantial plasticity remains in adult mammalian nervous system following injury and that neurological function is, to a certain degree, potentially amenable to pharmacological or other interventions, even relatively late after injury.

In summary, the present findings indicate that TRH, at doses as low as one one-hundredth of those previously shown to be effective in experimental spinal cord injury, significantly improve neurological recovery following experimental cervical spinal cord injury in the cat. At high doses, TRH treatment is effective even when administered 24 hrs following trauma. In view of recent studies showing that high doses of TRH are well tolerated, even in patients with a severe chronic neurological disorder affecting motor systems, the present studies provide further support for rapid institution of clinical trials with TRH in human spinal cord injury.

#### ACKNOWLEDGEMENTS

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#### FIGURE LEGEND

FIGURE 1. Effects of thyrotropin-releasing hormone or saline treatment on neurological recovery six weeks after cervical spine injury in cats. TRH-treatment significantly improved motor recovery in dose-related manner. TRH also significantly reduced late paralysis even when treatment was not administered until 24 hrs after injury. Points represent the sum of forelimb and hindlimb neurologic scores for individual animals; histograms represent median scores.



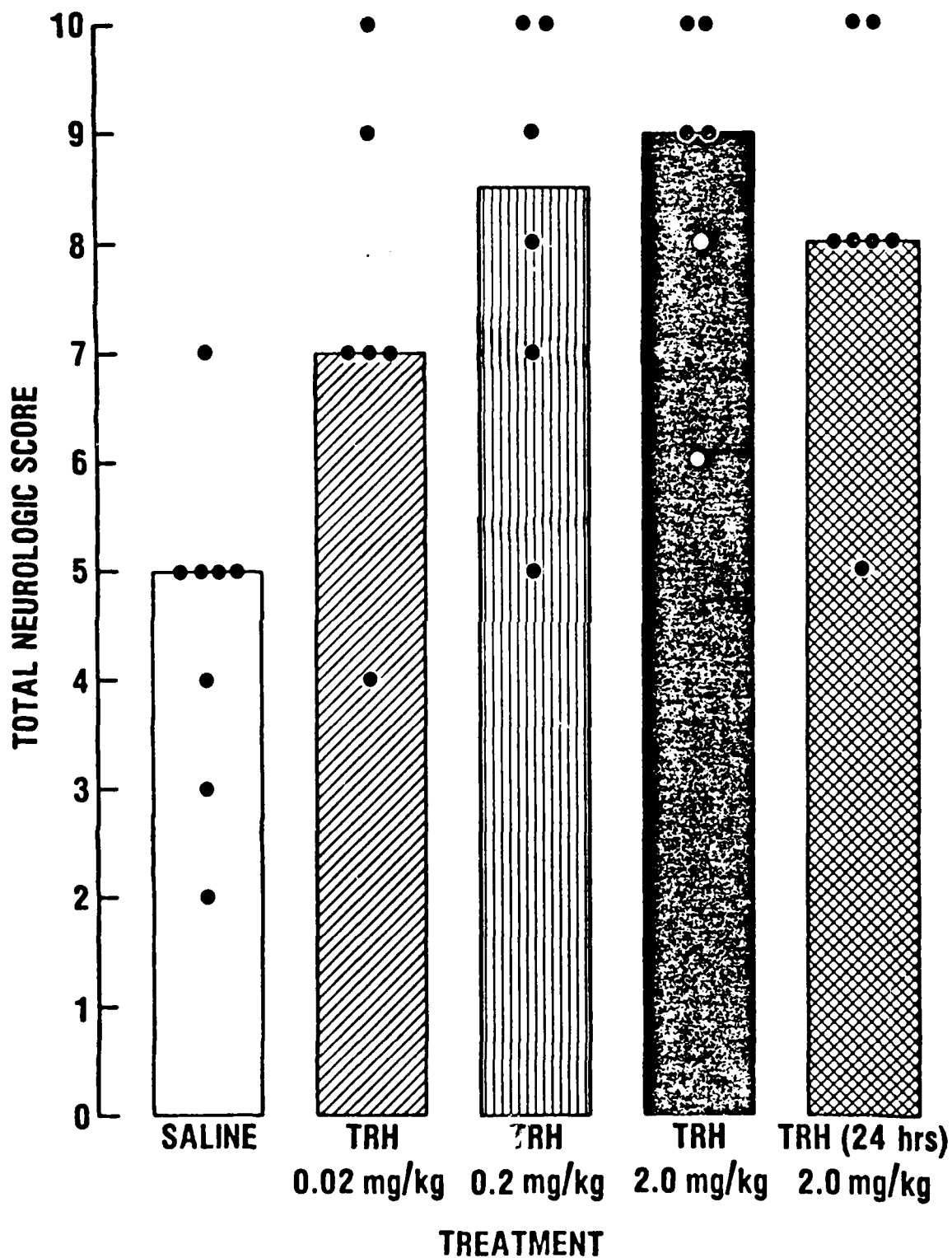


Figure 1.

#### REFERENCES

1. Ducker, T. B., G. W. Kindt, and L. G. Kempe. 1971. Pathological findings in acute experimental spinal cord trauma. J. Neurosurg. 35: 700-708.
2. Faden, A. I., T. P. Jacobs, E. Mougey, and J. W. Holaday. 1981. Endorphins in experimental spinal injury: therapeutic effect of naloxone. Ann. Neurol. 10: 326-332.
3. Faden, A. I., T. P. Jacobs, and J. W. Holaday. 1981. Opiate antagonist improves neurologic recovery after spinal injury. Science 211: 493-494.
4. Faden, A. I., T. P. Jacobs, and J. W. Holaday. 1982. Comparison of early and late naloxone treatment in experimental spinal injury. Neurology 32: 677-681.
5. Yarbrough, G. G. 1979. On the neuropharmacology of thyrotropin releasing hormone (TRH). Prog. Neurobiol. 12: 291-312.
6. Horita, A., M. A. Carino, and R. M. Chesnut. 1976. Influence of thyrotropin releasing hormone (TRH) on drug-induced narcosis and hypothermia in rabbits. Psychopharmacology (Berlin) 49: 57-62.
7. Holaday, J. W., R. J. D'Amato, and A. I. Faden. 1981. Thyrotropin-releasing hormone improves cardiovascular function in experimental endotoxic and hemorrhagic shock. Science 213: 216-218.
8. Faden, A. I., T. P. Jacobs, and J. W. Holaday. 1981. Thyrotropin-releasing hormone improves neurologic recovery after spinal trauma in cats. N. Engl. J. Med. 305: 1063-1067.

9. Faden, A. I., T. P. Jacobs, M. T. Smith, J. W. Holaday. (In press). Comparison of thyrotropin-releasing hormone (TRH), naloxone and dexamethasone treatments in experimental spinal injury. Neurology.
10. Sawin, C. T., J. M. Hershman, and I. J. Chopra. 1977. The comparative effect of  $T_4$  and  $T_5$  on the TSH response to TRH in young adult men. J. Clin. Endocrinol. Metab. 44: 273-278.
11. Chase, T. N., A. C. Woods, M. A. Lipton, and C. E. Morris. 1974. Hypothalamic releasing factors and Parkinson's disease. Arch. Neurol. 31: 55-56.
12. Engel, W. K., T. Siddique, and J. T. Nicoloff. 1983. Thyrotropin releasing hormone (TRH) acutely in amyotrophic lateral sclerosis (ALS) patients causes increased mobility and strength, lessened spasticity, shivering and tachypnea. Neurology 33(Suppl 2): 120-121.
13. Faden, A. I., T. P. Jacobs, G. P. Smith, B. A. Green, and J. A. Zivin. (In Press). Neuropeptides in spinal cord injury: comparative experimental models. Peptides.
14. Lux, W. E., Jr., G. Feuerstein, and A. I. Faden. 1983. Alteration of leukotriene  $D_4$  hypotension by thyrotropin releasing hormone. Nature 302: 822-824.
15. Lux, W. E., Jr., G. Feuerstein, and A. I. Faden. 1983. Thyrotropin-releasing hormone reverses the hypotension and bradycardia produced by leukotriene  $D_4$  in unanesthetized guinea pigs. Prostaglandins Leukotrienes Med. 10: 301-307.

16. Lux, W. E., Jr., G. Feuerstein, F. Snyder, and A. I. Faden. 1983. Effect of thyrotropin-releasing hormone on hypotension produced by platelet-activating factor. Circ. Shock 10: 262.
17. Marsden, C. A., G. W. Bennett, J. Irons, R. F. T. Gilbert, and P. C. Emson. 1982. Localization and release of 5-hydroxytryptamine, thyrotropin releasing hormone and substance P in rat ventral spinal cord. Comp. Biochem. Physiol. 72C: 263-270.
18. Boyer, C. E., and B. R. Cooper. 1982. Thyrotropin releasing hormone in cat spinal cord - excitatory effects, regional distribution and effects of chronic spinal cord lesions. Fed. Proc. 41: 1080.
19. Ogawa, N., Y. Yamawaki, H. Kuroda, T. Ofuji, E. Itoga, and S. Kito. 1981. Discrete regional distribution of thyrotropin releasing hormone (TRH) receptor binding in monkey central nervous system. Brain Res. 205: 169-174.
20. Schultzberg, M., T. Hökfelt, and J. M. Lundberg. 1982. Coexistence of classical transmitters and peptides in the central and peripheral nervous systems. Brit. Med. Bull. 38: 309-313.
21. Nicholl, R. A. 1978. The action of thyrotropin releasing hormone, substance P and related peptides on frog spinal motoneurons. J. Pharmacol. Exp. Ther. 207: 817-824.
22. Phillis, J. W., and J. R. Kirkpatrick. 1979. Actions of various gastrointestinal peptides on the isolated amphibian spinal cord. Can. J. Physiol. Pharmacol. 51: 887-899.

23. Ono, H., and H. Fukuda. 1982. Ventral root depolarization and spinal reflex augmentation by a TRH analog in rat spinal cord. Neuropharmacology 21: 739-744.
24. Feringa, E. R., R. D. Johnson, and J. S. Wendt. 1975. Spinal cord regeneration in rats after immunosuppressant treatment: theoretic considerations and histologic results. Arch. Neurol. 32: 676-683.
25. Demopoulos, H. B., E. S. Flamm, M. L. Seligman, J. A. Mita-mura, and J. Ransohoff. 1979. Membrane perturbations in central nervous system injury: theoretical basis for free radical damage and a review of the experimental data. In Neural Trauma. A. J. Popp et al., editors. Raven Press, New York.
26. Zivin, J. A., J. L. Doppman, J. L. Reid, M. L. Toppaz, J. M. Saavedra, I. J. Kopin, and D. M. Jacobowitz. 1976. Biochemical and histochemical studies of biogenic amines in spinal cord trauma. Neurology 26: 99-107.
27. Faden, A. I., T. P. Jacobs, and J. W. Holaday. 1982. Neuropeptides and spinal cord injury. In Regulatory Peptides: From Molecular Biology to Function. E. Costa, Trabucchi M, editors. Raven Press, New York.
28. Stokes, B. T., P. Fox, and G. Hollinden. 1983. Extracellular metabolites: their measurement and role in the acute phase of spinal cord injury. In The Proceedings of the Fifth Neuro-trauma Conference. J. Jane, editor.

29. Sandler, A. N., and C. H. Tator. 1967. Review of the effect of spinal cord trauma on the vessels and blood flow in the spinal cord. J. Neurosurg. 5: 638-646.
30. Braughler, J. M., and E. D. Hall. 1982. Correlation of methylprednisolone levels in cat spinal cord with its effect on  $(Na^{+} + K^{+})$ -ATPase, lipid peroxidation, and alpha motor neuron function. J. Neurosurg. 56: 838-844.
31. Albin, M. S., R. J. White, D. Yashon, and L. S. Harris. 1969. Effects of localized cooling on spinal cord trauma. J. Trauma 9: 1000-8.
32. Faden, A. I., J. M. Hallenbeck, and C. Q. Brown. 1982. Treatment of experimental stroke: comparison of naloxone and thyrotropin releasing hormone. Neurology 10: 1083-1087.

APPENDIX B

MEGADOSE CORTICOSTEROID THERAPY FOLLOWING  
EXPERIMENTAL TRAUMATIC SPINAL INJURY

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# ABSTRACT

Corticosteroids are routinely used in the treatment of spinal trauma, although neither experimental nor clinical support for their use is persuasive. Recently there have been claims that extremely high doses ("megadose") of corticosteroids (equivalent to 15 - 30 mg/kg of methylprednisolone) improve neurological recovery when compared against traditional steroid doses. We therefore compared megadose dexamethasone and methylprednisolone therapy against saline following traumatic cervical spinal injury in the cat. During six weeks post-injury, neurological recovery did not differ significantly between corticosteroid-treated and control animals. Moreover, histopathological changes in the spinal cord were similar in methylprednisolone- and saline-treated cats. Corticosteroid-treated animals had a higher mortality rate than control animals, with predominant cause of death being neurogenic pulmonary edema. Thus, megadose corticosteroid treatment does not improve neurological recovery in this experimental model of spinal injury, and is associated with increased mortality.

Key Words: megadose corticosteroids, dexamethasone, methylprednisolone, traumatic spinal injury



Corticosteroids are routinely and widely utilized in the treatment of spinal cord injury despite the fact that they have never been demonstrated to be effective in human spinal trauma. Rather, institution of steroid therapy has been based on theoretical justifications for their use (e.g., the effects on lysosomal membranes, tissue edema, lipid peroxidation), combined with experimental reports showing beneficial effects in some animal injury models. However, the theoretical justifications are of uncertain relevance since the pathophysiologic mechanisms underlying acute spinal cord injury are not well understood.<sup>7</sup> Moreover, the experimental literature supporting the use of corticosteroids for spinal cord injury is controversial; whereas many investigators have shown beneficial actions for corticosteroids in certain models,<sup>1,4,10,18,21,25,34</sup> others have failed to show significant improvement of neurological recovery.<sup>11,14,22,23,28</sup>

These conflicting experimental reports have resulted in the utilization of ever increasing steroid doses. Most recently it has been suggested that even high dose corticosteroid therapy may be insufficient. Based in part on experimental work showing that so-called "megadose" methylprednisolone (15 - 30 mg/kg) significantly inhibits lipid peroxide formation and enhances  $\text{Na}^+ - \text{P}^+ - \text{ATPase}$  activity,<sup>2,8</sup> several laboratories have utilized methylprednisolone doses in this range and have reported improvement in either physiologic variables or functional recovery.<sup>18,25,34</sup> In contrast, another group has found no beneficial effect for "megadose" dexamethasone at a dose of 20 mg/kg.<sup>11</sup> Some of these inter-laboratory differences may be due to differences in experimental design such as species, injury method, steroid preparation or treatment dose schedule.

To address some of these questions, we have examined the effects of megadose therapy with either dexamethasone or methylprednisolone following traumatic spinal injury in the cat, a species previously utilized to show the beneficial effects of megadose methylprednisolone.

#### MATERIALS AND METHODS

Specific pathogen-free (SPF) cats (Liberty Labs) weighing  $3.0 \pm 0.25$  kg were used. After anesthesia with sodium pentobarbital (25 mg/kg, IV) the animals were intubated, paralyzed with gallamine triethiodide (4 mg/kg/h) and artificially ventilated with a Harvard respirator. Arterial blood gas values were maintained in the normal range through use of IV sodium bicarbonate and respirator adjustments. Temperature was maintained at  $38^{\circ}\text{C}$  with a feedback thermoregulating unit (Yellow Springs). Blood pressure was continuously recorded through a femoral artery catheter connected to a pressure transducer and physiograph (Narco Bio-Systems). A catheter in the femoral vein permitted infusion of drugs. After the animal's head was fixed in a stereotaxic unit (David Kopf), a ligature was placed through the supraspinous ligament between  $T_2$ - $T_3$  to stabilize the cervical vertebrae in a horizontal plane. Under sterile conditions, a laminectomy was performed to expose the  $C_7$  spinal segment. With the dura intact, the spinal cord was traumatized utilizing a modification of the Allen method<sup>14</sup> in which a 20 gm weight was dropped a distance of 40 cm (Study I,  $n = 11$ ) or 30 cm (Study II,  $n = 21$ ) through a guide tube onto a  $10\text{ mm}^2$  impact plate. The injury variables (600 or 800 g-cm) were chosen from pilot and earlier studies showing that they produced moderate to severe spastic quadriparesis in untreated control animals at six weeks

following injury. Treatment was initiated one h post-injury with physiologic saline (Study I, n = 4; Study II, n = 11) or corticosteroids (dexamethasone in Study I, n = 7; methylprednisolone sodium succinate in Study II, n = 10). Methylprednisolone (kindly provided by Upjohn) was administered t.i.d. in the following manner: trauma day, 30 mg/kg total (i.e., 15 mg/kg, IV, 7.5 mg/kg x 2, IM); day 2, 15 mg/kg total, IM; day 3, 15 mg/kg; day 4, 12 mg/kg; day 5, 9 mg/kg; day 6, 7.5 mg/kg; day 7, 6 mg/kg; day 8, 4.5 mg/kg; day 9, 3.0 mg/kg. Dexamethasone (Carter-Glogau) was administered as a 2 mg/kg bolus followed by 2 mg/kg/h over six h (total = 14 mg/kg).

The rationale for Study I was based on earlier studies from our laboratory showing that high dose (2.5 mg/kg) dexamethasone therapy was ineffective in traumatic cervical spinal injury (600 g-cm).<sup>14</sup> Use of the higher injury variables was an attempt to maximize potential differences between control and treatment animals; however, this study was aborted because of the extremely high mortality rate in dexamethasone-treated animals (vide infra). Study II employed injury variables previously employed in our laboratory and utilized methylprednisolone instead of dexamethasone to enable comparisons with other published studies.<sup>18,25</sup>

Following treatment, the catheters were removed, animals were given 600,000 units of procaine bicillin, IM, then placed in a temperature controlled cage. When the animals were thermoregulated, they were returned to their home cages. Neurologic function was evaluated weekly by a neurologist who was unaware of each animal's treatment group. A modification of the five-point ordinal scale originally developed by Tarlov was used to score forelimb and hindlimb function as follows: 0 = absence of

voluntary movement; 1 = spontaneous movement, but inability to support weight; 2 = ability to support weight but unable to walk; 3 = ability to walk but with marked spasticity and/or ataxia; 4 = ability to run but with mild spasticity or ataxia; 5 = normal motor function. A total functional neurologic score was obtained by adding the hindlimb and forelimb scores.

At six weeks post-injury the animals were euthanized with sodium pentobarbital. Spinal cords were removed and fixed in glutaraldehyde for seven days. Spinal cord segments C<sub>5</sub>-T<sub>2</sub> were sliced in cross-sections at 2 mm intervals allowing careful measurement of the craniocaudal extent of lesions present. The cross-sectional slices were then dehydrated in graded alcohols, embedded in paraffin and cut in 5  $\mu$  sections with a rotary microtome. Sections were stained with hematoxylin, eosin and luxol-fast blue and studied with a light microscope. The area of demyelination at the injury site was measured using an ocular micrometer. The total volume of injury was calculated using the formula  $V = L \times X \times Y$  where V is volume; L is craniocaudal extent of injury; X and Y are the sides of the rectangle approximated by the cross-sectional area of injury (Figure 1). The volume of injury was scored according to the following:

<u>Score</u>	<u>Injury Volume (mm<sup>3</sup>)</u>
1	81 - 100
2	61 - 80
3	41 - 60
4	21 - 40
5	0 - 20

A complete necropsy was performed on all animals that died prior to the six week follow-up period. Gross and histopathologic examinations of

tissues from these cats were performed to identify pathologic changes and the cause of death.

Blood pressure changes between groups were analyzed with repeated measurement analysis of variance. Neurologic and pathologic scores were compared using the Mann-Whitney rank sum test. Correlations between neurologic and pathologic scores were evaluated using the Spearman rank correlation test. Fisher's exact probability test was used to test survival rates between groups. A p value < 0.05 was considered statistically significant.

#### RESULTS

The spinal cords displayed typical traumatic lesions after the standardized trauma: many were cavitated; all showed degrees of gliosis, demyelination and axonal reaction. Remaining myelin was concentrated at the periphery of the cord. Minimal cellular reaction was observed. Histopathology scores did not differ significantly between corticosteroid-treated and control animals (Table 1) ( $p > 0.05$ , Mann-Whitney rank sum test).

Trauma caused a transient increase in mean arterial pressure followed by a gradual decline over the next 60 min from  $129 \pm 6$  to  $87 \pm 7$  mmHg (mean  $\pm$  SEM). Although treatment with methylprednisolone caused a slight decrease in mean arterial pressure within 30 min after injection (Figure 2), there were no significant differences between groups at any time during the four h monitoring period (repeated measures ANOVA,  $F = 0.14$ ,  $p > 0.05$ ).

Neurologic function was not significantly different between animals treated with methylprednisolone and in control animals treated with saline

(Figure 3) at any time during the six week follow-up period (Table 2) ( $p > 0.05$ , Mann-Whitney rank sum test).

In both studies (Table 3), the mortality rate was higher in corticosteroid-treated than in control animals (72% vs. 0% in Study I, 40% vs. 28% in Study II). These differences were not significant ( $p = 0.07$ ), possibly due to the small number of animals in each group.

All of the deaths in the two studies occurred two to five days post-trauma. The principal gross pathological observation was the development of pulmonary edema, with wet heavy pulmonary parenchyma, mediastinal edema and pleural effusion of copious amounts of serosanguineous fluid (Figure 4). Pulmonary histopathological changes were consistent with the gross findings and included alveolar capillary congestion, dilated lacteals, and protein and fibrin aggregates within alveolar spaces (Figure 5).

#### DISCUSSION

The present findings demonstrate that megadose corticosteroid therapy fails to reduce either the neurological dysfunction or the histopathological changes produced by traumatic cervical spinal cord injury in the cat. Two independent studies were performed. The first evaluated the effect of dexamethasone (14 mg/kg, IV, over six h) in a severe cervical injury model (800 g-cm). This study was aborted prematurely because of the very high mortality rate of the dexamethasone-treated animals (five of seven). The second study utilized a somewhat less severe injury model (600 g-cm) and employed methylprednisolone at doses previously shown to be effective in in vivo and in vitro studies;<sup>2,18,25,34</sup> rationale for the changes in methodology in Study II resulted in part from the excessive mortality rate in Study I and in part from a desire to make the model similar to that

used for studies investigating the therapeutic effects of naloxone<sup>12</sup> and thyrotropin-releasing hormone.<sup>14</sup> Use of methylprednisolone rather than dexamethasone in this study was necessitated by the question of potentially different rates of entry into the central nervous system by the two steroid preparations, as well as the previous reports of the beneficial effects of methylprednisolone after spinal injury in the cat.<sup>2,25,34</sup> As in the dexamethasone study, however, methylprednisolone treatment was associated with a trend toward higher mortality and failed to improve neurological function or to reduce histopathological changes. Although the mortality rates associated with megadose corticosteroid therapy (72% and 40%, respectively, for Studies I and II) just failed to reach significance as compared with saline controls ( $p = 0.07$ ), they are significantly higher than those of naloxone- (mortality rate = 12%,  $p < 0.05$ )<sup>12</sup> or TRH- (mortality rate = 0%,  $p < 0.05$ )<sup>14</sup> treated animals in other studies using an identical spinal injury model. Moreover, the mortality rates with corticosteroid therapy in the present studies are similar to those previously reported with this model utilizing high dose dexamethasone (mortality rate = 40%).<sup>14</sup> Mean arterial blood pressure, enhanced with other treatments which improve neurological recovery in this model,<sup>12,14</sup> was not significantly affected by methylprednisolone treatment.<sup>7</sup>

The principal cause of death in both studies, as in previous studies utilizing this model,<sup>14</sup> appeared to be pulmonary edema. We believe this to be neurogenic in origin since no non-neurogenic causes were found to account for the edema. Death from neurogenic pulmonary edema similarly has been reported in humans after cervical spinal injury.<sup>26</sup> Other neurologic conditions associated with induction of pulmonary edema include

cranial trauma, cerebral hemorrhage, epilepsy, elevated intracranial pressure and brain tumors.<sup>5,9,17,27,30,31,33</sup> The precise pathophysiologic mechanisms involved are unknown, but the major hypotheses suggest a transient systemic and pulmonary hypertension and/or a loss of pulmonary capillary integrity.<sup>24,32</sup>

An increased tendency for complications without beneficial effects has been noted following high dose corticosteroid treatment in humans for head injury,<sup>3,6,20</sup> spinal injury<sup>29</sup> or brain tumor.<sup>19</sup> A recent controlled trial comparing high (15 mg/kg) and low dose methylprednisolone therapy in human spinal injury showed no beneficial effect of the high dose on neurological recovery; yet infection rates were higher and there was a trend suggesting higher mortality from cardiopulmonary complications.<sup>29</sup>

The lack of effect of megadose corticosteroid therapy in this study and in the study by Eidelberg<sup>11</sup> contrasts with the findings from several other laboratories. Inter-laboratory differences have been characteristic of experimental work in spinal cord injury and may result from methodological differences between laboratories. However, most of the previous positive studies were performed in the cat, as in the present experiments, and utilized similar doses and dose schedules for methylprednisolone. Moreover, neither method of injury nor site of injury appears likely to explain the reported differences. On the other hand, the reported beneficial effects of megadose methylprednisolone and megadose dexamethasone in the Rhesus monkey have been limited (albeit statistically significant).<sup>18</sup> In contrast to the lack of effect of megadose corticosteroid treatment in our cat model, treatment with either naloxone<sup>12,13,15,16</sup> or thyrotropin-releasing hormone<sup>14,16</sup> have resulted in dramatically improved



neurological recovery in this model. Furthermore, both naloxone and TRH therapy have proved superior to high dose dexamethasone treatment when these therapies have been directly compared.<sup>16</sup>

Although the effect of megadose or high dose corticosteroid therapy on neurological recovery remains questionable, the effects of this therapy on physiologic variables appear to be much more persuasive. For example, megadose methylprednisolone therapy has been shown to significantly improve spinal cord blood flow,<sup>34</sup> recovery of extracellular calcium,<sup>34</sup> enhancement of spinal cord  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  activity<sup>2</sup> and attenuation of lipid peroxide formation.<sup>2</sup> However, in view of the present as well as earlier functional studies, such physiological actions may not relate directly to neurological recovery. Moreover, even if megadose corticosteroid therapy was associated with some improvement of neurologic recovery, the increase in morbidity and mortality with such doses as observed in the present and other studies suggests caution with regard to their potential utilization for human spinal cord injury.

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TABLE 1  
Histopathology Scores for Methylprednisolone-Treated  
Cats After Cervical-Spine Injury

HISTOPATHOLOGY	SALINE	METHYLPREDNISOLONE
SCORE	(n = 8)	(n = 6)
5	—	—
4	X	X
3	XXX	XX
2	XX	—
1	XX	XXX

X - represents scores for individual animals.

TABLE 2

Effects of Methylprednisolone Treatment on Median  
Neurologic Score After Spinal Cord Injury

Weeks After Injury	<u>METHYLPREDNISOLONE</u>			<u>SALINE</u>		
	Forelimb	Hindlimb	Total	Forelimb	Hindlimb	Total
1	2	1	3	3	0.5	3.5
2	2.5	2	4.5	3	1.5	4.5
3	3	2	5	3	1.5	4.5
4	3	3	6	3	1.5	4.5
5	3	3	6	3	2	5
6	3	2.5	5.5	3	2	5

TABLE 3

Effects of Corticosteroid Treatment on Survival  
in Experimental Spinal Cord Injured Cats

## A. STUDY I (800 g-cm)

Treatment	Death	Survival	% Survival
Saline	0	4	100%
Dexamethasone	5	2	28%

## B. STUDY II (600 g-cm)

Treatment	Death	Survival	% Survival
Saline	3	8	72%
Methylprednisolone	4	6	60%

#### FIGURE LEGENDS

- Fig. 1 This cross section of cervical spinal cord is characteristic of severe injury resulting from the standardized trauma. Cavities are produced which are located predominantly in white matter. Remaining stainable myelin is seen in the periphery of the cord (Luxol fast blue X10). Arrows demarcate the total area of injury (see text).
- Fig. 2 Effects of methylprednisolone (n = 6) or saline (n = 8) treatment on mean arterial pressure (MAP) after traumatic injury (600 g-cm) to the cervical spinal cord. No significant differences were observed between the groups. Points represent mean values  $\pm$  S.E.M.
- Fig. 3 Effects of methylprednisolone (n = 6) or saline (n = 8) treatment on neurologic recovery six weeks after cervical-spinal cord injury. Points represent the sums of forelimb and hindlimb neurologic scores for individual animals; histograms represent median scores. No significant differences were observed between the groups.
- Fig. 4 A pleural effusion is also evident in this specimen from a cat with typical post-traumatic pulmonary edema. Grossly, the lungs appear edematous, swollen and wet.
- Fig. 5 This photomicrograph of lung illustrates severe pulmonary edema. Alveoli are filled with an eosinophilic, proteinaceous fluid. Scattered macrophages are present (Hematoxylin and eosin X400).

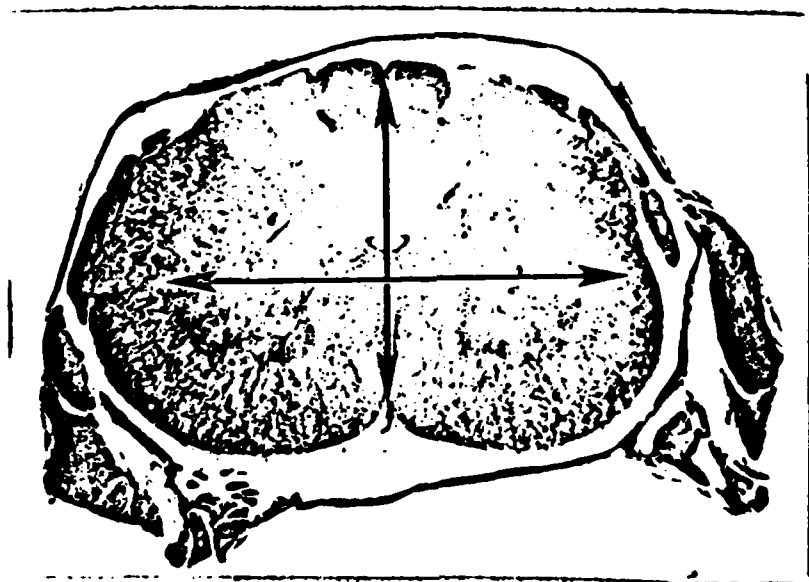


Figure 1

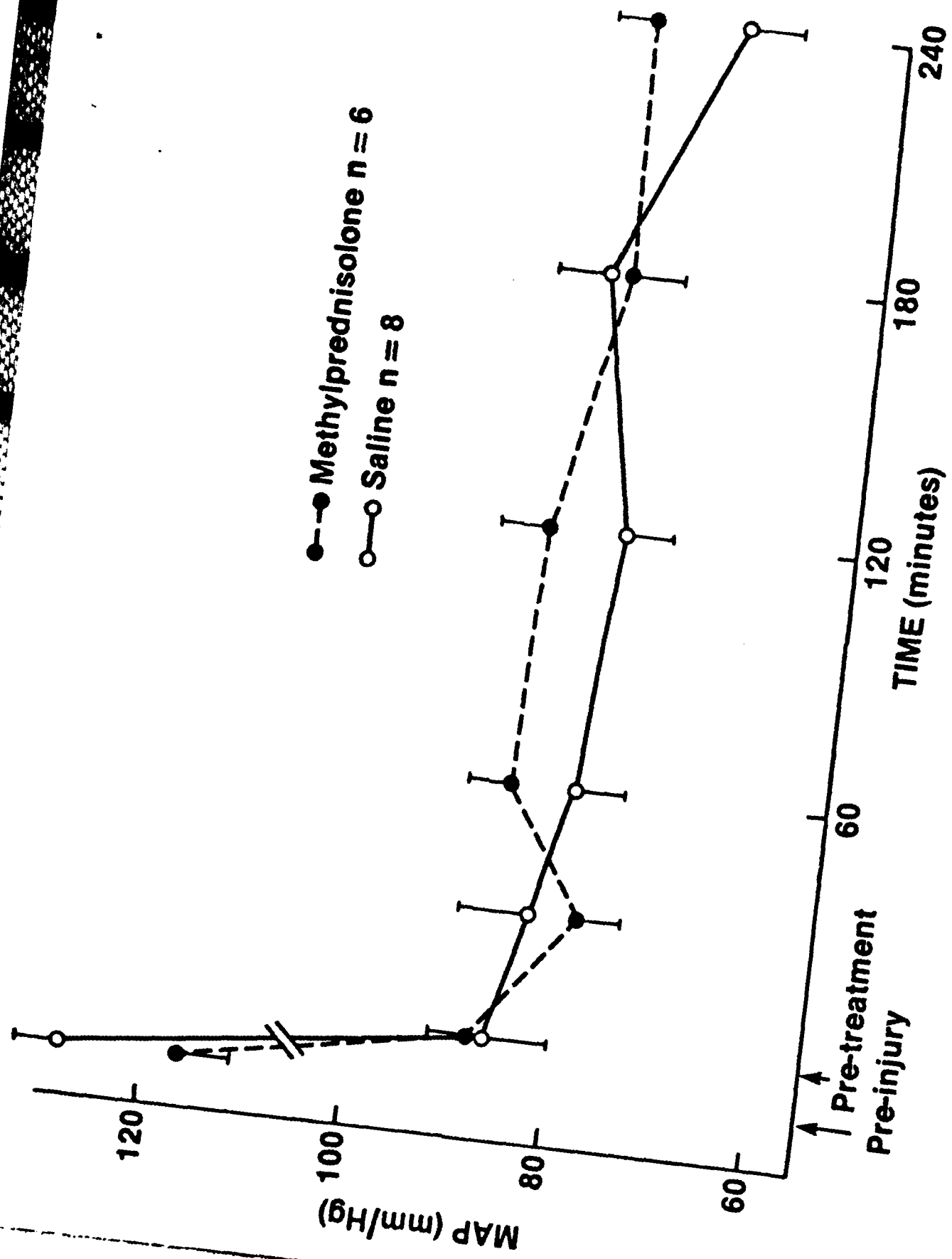


Figure 2

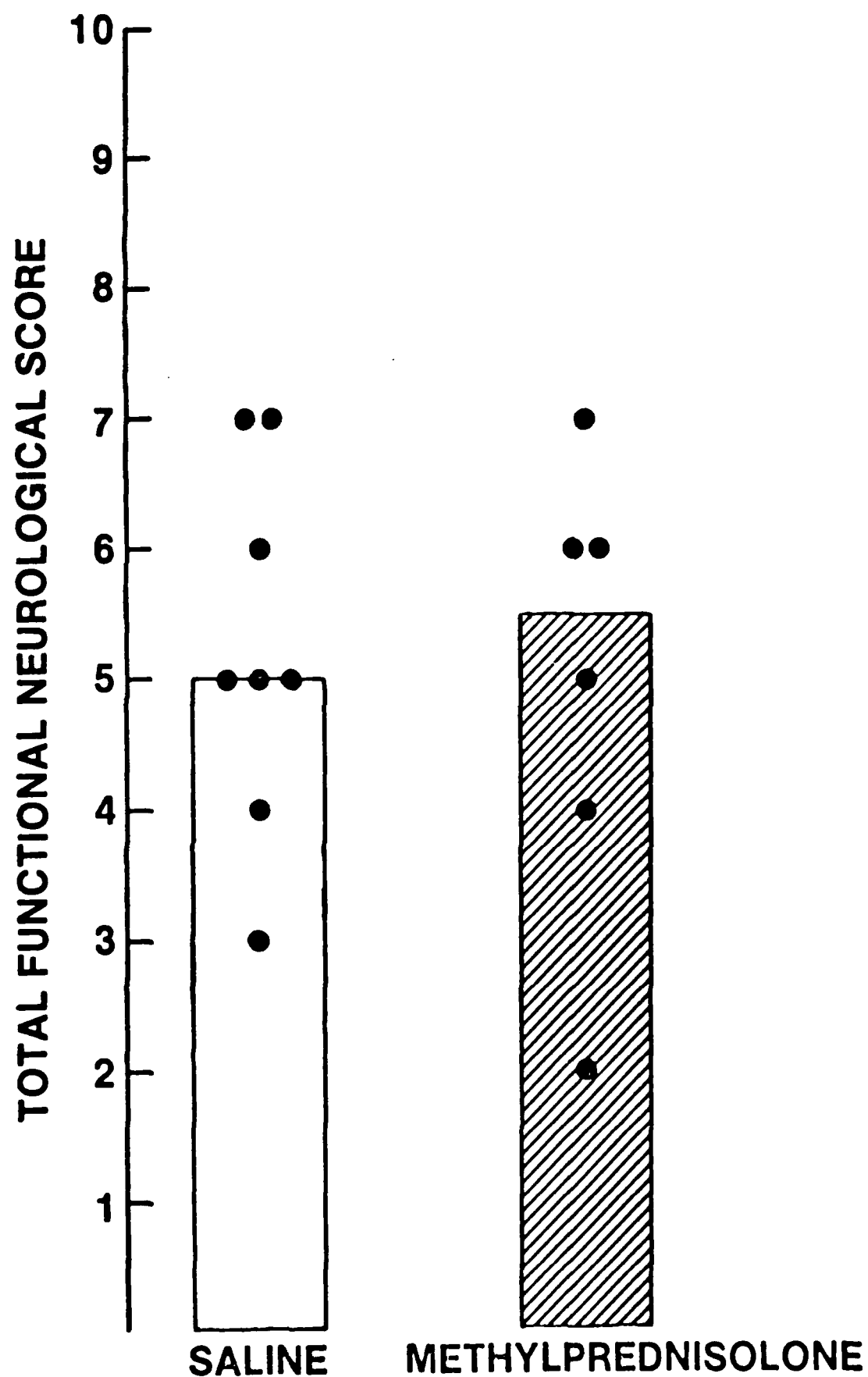


Figure 3



Figure 4 ↓

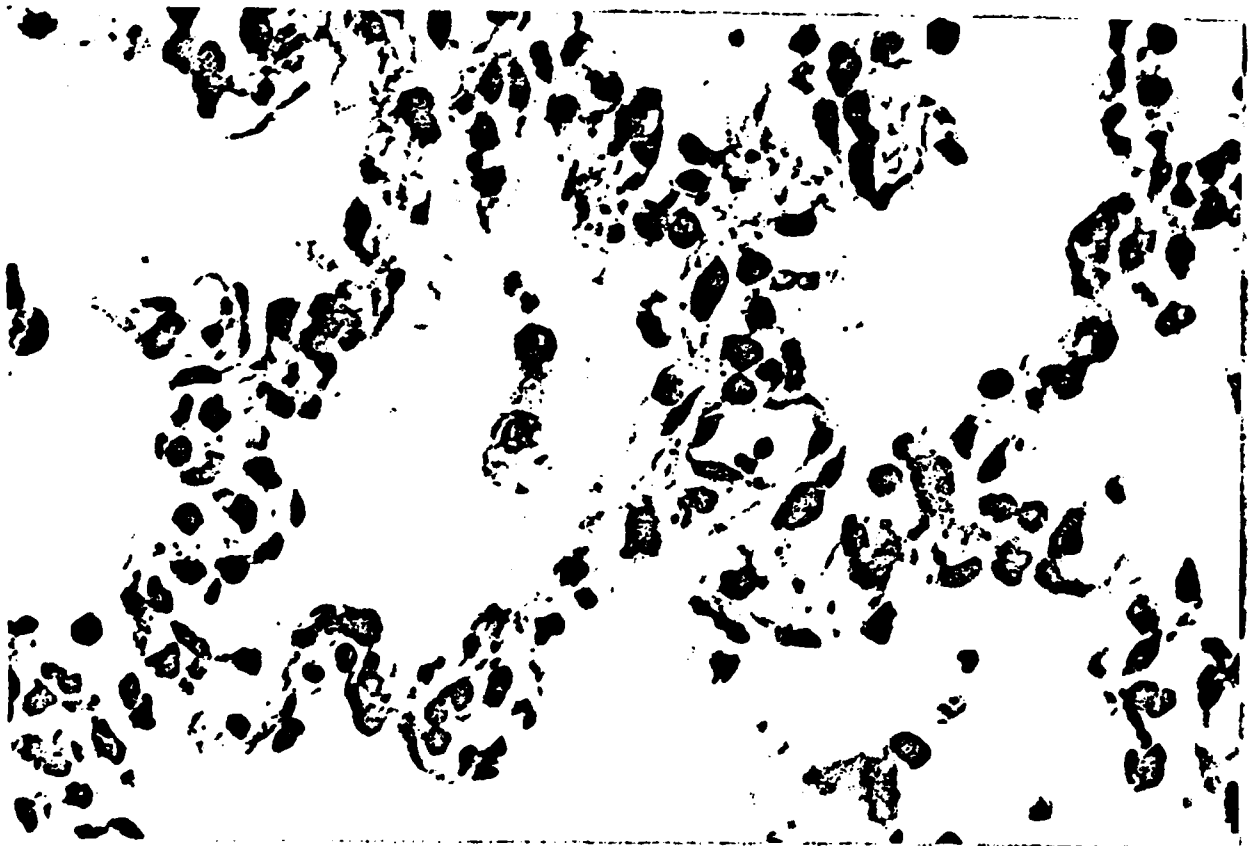


Figure 5 ↑

#### REFERENCES

1. Black P, Markowitz RS: Experimental spinal cord injury in monkeys: comparison of steroids and local hypothermia. Surg Forum 22:409-411, 1971
2. Braughler JM, Hall ED: Correlation of methylprednisolone levels in cat spinal cord with its effect on  $(Na^{+} + K^{+})$ -ATPase, lipid peroxidation, and alpha motor neuron function. J Neurosurg 56:838-844, 1982
3. Broakman R, Schouten HJA, Blaauw-van Dishoeck M, et al: Megadose steroids in severe head injury, results of a prospective double-blind trial. J Neurosurg 58:326-330, 1983
4. Campbell JB, DeCrescito V, Tomasula JJ, et al: Effects of antibrinolytic and steroid therapy on the contused spinal cords of cats. J Neurosurg 40:726-733, 1974
5. Carlson RW, Schaeffer RC, Michaels SG, et al: Pulmonary edema following intracranial hemorrhage. Chest 6:731-734, 1979
6. Cooper PR, Moody S, Clark WK, et al: Dexamethasone and severe head injury: a prospective double-blind study. J Neurosurg 51:307-316, 1979
7. de la Torre JC: Spinal cord injury: review of basic and applied research. Spine 6:315-335, 1981.
8. Demopoulos HB, Flamm ES, Pietronigro DD, et al: The free radical pathology and the microcirculation in the major central nervous system disorders. Acta Physiol Scand Suppl 492:91-119, 1980
9. Ducker TB: Central nervous system pressure and pulmonary edema. Trans Am Neurol Assoc 92:225-229, 1967

10. Ducker TB, Hamit HF: Experimental treatments of acute spinal cord injury. J Neurosurg 30:693-697, 1969
11. Eidelberg E, Staten E, Watkins CJ, et al: Treatment of experimental spinal cord injury in ferrets. Surg Neurol 6:243-246, 1976
12. Faden AI, Jacobs TP, Holaday JW: Comparison of early and late naloxone treatment in experimental spinal injury. Neurology 32:677-681, 1982
13. Faden AI, Jacobs TP, Holaday JW: Opiate antagonist improves neurologic recovery after spinal injury. Science 211:493-494, 1981
14. Faden AI, Jacobs TP, Holaday JW: Thyrotropin-releasing hormone improves neurologic recovery after spinal trauma in cats. N Engl J Med 305:1063-1067, 1981
15. Faden AI, Jacobs TP, Mougey E, et al: Endorphins in experimental spinal injury: Therapeutic effect of naloxone. Ann Neurol 10:326-332, 1981
16. Faden AI, Jacobs TP, Smith MT, et al: Comparison of thyrotropin-releasing hormone (TRH), naloxone and dexamethasone treatments in experimental spinal injury. Neurology 33:673-678, 1983
17. Felman AH: Neurogenic pulmonary edema: Observations in six patients. Ann J Roentgenol Radium Ther Nucl Med 112:393-398, 1971
18. Green BA, Kahn T, Klose KJ: A comparative study of steroid therapy in acute experimental spinal cord injury. Surg Neurol 13:91-97, 1980
19. Green SB, Byar DP, Walker MD, et al: Comparisons of carmustine, procarbazine, and high-dose methylprednisolone as additions to surgery and radiotherapy for the treatment of malignant glioma. Cancer Treatment Reprints 67:121-132, 1983

20. Gudeman SK, Miller JD, Becker DP: Failure of high dose steroid therapy to influence intracranial pressure in patients with severe head injury. J Neurosurg 51:301-306, 1979
21. Hansebout RR, Kuchner EF, Romero-Sierra C: Effects of local hypothermia and of steroids upon recovery from experimental spinal cord compression injury. Surg Neurol 4:531-536, 1975
22. Hedeman LS, Sil R: Studies in experimental spinal cord trauma. Part 2: Comparison of treatment with steroids, low molecular weight dextran, and catecholamine blockade. J Neurosurg 40:44-51, 1974
23. Hoerlein BF, Redding RW, Hoff EJ, et al: Evaluation of dexamethasone, DMSO, mannitol and solcoseryl in acute spinal cord trauma. J Amer Anim Hosp Assoc 19:216-226, 1983
24. Kosnik EJ, Paul SE, Rosel CW, et al: Central neurogenic pulmonary edema: with a review of its pathogenesis and treatment. Child's Brain 3:37-47, 1977
25. Means ED, Anderson DK, Waters TR, et al: Effect of methylprednisolone in compression trauma to the feline spinal cord. J Neurosurg 55:200-208, 1981
26. Meyer GA, Berman IR, Dote DB, et al: Hemodynamic responses to acute quadriplegia with or without chest trauma. J Neurosurg 34:168-177, 1971
27. Milley JR, Nugent SK, Rogers MC: Neurogenic pulmonary edema in childhood. J Pediatrics 94:706-709, 1979
28. Parker AJ, Smith CW: Functional recovery from spinal cord trauma following dexamethasone and chlorpromazine therapy in dogs. Res Vet Sci 21:246-247, 1976

29. Report of the National Collaborative Spinal Cord Study. In Review.
30. Simmons FL, Martin AM Jr, Heisterkamp CA III, et al: Respiratory insufficiency in combat casualties II. Pulmonary edema following head injury. Ann Surg 39:170-179, 1969
31. Terrence CF, Rao GR, Perper JA: Neurogenic pulmonary edema in unexpected, unexplained death of epileptic patients. Ann Neurol 9: 458-464, 1981
32. Theodore J, Robin ED: Speculations on neurogenic pulmonary edema. Am Rev Resp Dis 113:405-410, 1976
33. Wersman SJ: Edema and congestion of the lungs resulting from intracranial hemorrhage. Surgery 6:722-726, 1939
34. Young W, Flamm ES: Effect of high-dose corticosteroid therapy on blood flow, evoked potentials and extracellular calcium in experimental spinal injury. J Neurosurg 57:667-673, 1982

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